at concentrations as low as 50 mM. (c) Arginine has marked geometric concentration dependence trend in REA as well as Th-T. Polyamines have no noticeable effect on stability of lysozyme but profound effect on Th-T. (d) Trehalose reveals a different concentration profile than Arginine, thus pointing to difference in mode of action.

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Stacked Sheets and Solenoids: Implications of Polymorphic Amyloid Structures of the Fungal Prion HET-s

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Prions are aberrantly folded infectious proteins that propagate by inducing the refolding of normal proteins. Prions generally form amyloids: chemically homogeneous, fibrillar protein aggregates. Amyloids are associated with many diseases including Alzheimer's, type II diabetes, and more specifically to prions, Creutzfeldt-Jakob disease and bovine spongiform encephalopathy (''mad cow disease''). Despite this variety, all amyloids share a common cross-β structural motif, β-strands running perpendicular to a central fiber axis. Data from tissue extracted amyloids and studies of short amyloidogenic peptides have led some to hypothesize that all amyloids have a generic amyloid fold consisting of stacks of β-sheets. However, structural models of Alzheimer's related Aβ-amyloid and diabetes related IAPP amyloid indicate that amyloid structure is more diverse. Studies of brain-derived and recombinant prion protein, PrP, show that the generic amyloid fold shows only marginal biological activity while a β-solenoid fold is highly pathogenic.

We have looked at HET-s(218-289), the prion forming domain of a functional prion in the fungus *Podospora anserina*. It has been determined by solid state NMR that under physiological conditions, HET-s(218-289) fibrilizes into a β-solenoid fold. Others have shown that when fibrilized under low pH conditions, a non-functional polymorph is formed. We have determined by X-ray fiber diffraction that at low pH, proteolysis leads to the formation of stacked β-sheet amyloids. These amyloids can propagate the generic fold onto undegraded HET-s(218-289), though only under non-physiological conditions. These results indicate that the biological activity of HET-s(218-289) is intimately tied to its specific amyloid structure and that short amyloidogenic fragments may not adequately reproduce the interactions of larger prion domains.

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Evaluation of the Amyloid Fibril Stability

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Amyloid fibrils are associated with many neurodegenerative diseases.1-3 Being formed from proteins unrelated functionally, amyloid fibrils share a common cross-β core structural motif and are considered to be an extraordinarily stable and energetically most favorable form of proteins.4,5 Recently we reported that temperature and salinity variations result in a substantial melting of the fibril core of mature apo-α-lactalbumin fibrils and in a spontaneous refolding to a different fibril polymorph.6 An important question to address is whether the described phenomenon is relevant to other protein fibrils.

Our current findings indicate that a small pH change initiates the spontaneous transformation of insulin fibrils from one polymorph to another. Double-fiber-type fibrils with reverse VCD (vibrational circular dichroism) chirality form at pH 1.5, split into two separate proto-fibrils when the pH increases to 2.5 and intertwine to form left-handed twisted fibril polymorphs with normal VCD chirality. Using deep UV resonance Raman spectroscopy we demonstrate that the change of tyrosine local environments is taking place upon fibril spontaneous inter-conversion. At the same time, the fibril core has the same structure for both fibril polymorphs indicating that the pH-driven polymorphism of insulin fibrils is associated most probably with surface charge properties of protofibrils and the way they intertwine.7

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Implications Of *Cis* Interactions Between Expanded Polyglutamine and the Proline Rich C-Terminal Domain of Huntingtin Exon 1 For the Loss- Versus Gain-Of-Function Models of Huntington's Disease

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At least nine neurodegenerative disorders, including Huntington's disease (HD), have been associated with expanded polyglutamine (polyQ) tracts. Proteolytic products of these proteins form neuronal intranuclear inclusions. There is growing interest in the role of naturally occurring flanking sequences and persistent controversy regarding their role as gatekeepers against or enhancers of polyQ aggregation. In particular, previous studies have shown that the 17-residue N-terminal segment (N17) of huntingtin (htt) exon 1 acts as gatekeepers, although controversy lingers with regards to this finding. Here, we focus on the interactions in *cis* between polyQ and the proline rich C-terminal domain (C38) of htt exon 1. This domain possesses two polyproline stretches (polyP) that appear to interact with profilin, which inhibits htt aggregation in cell culture studies.

Through the use of atomistic Monte Carlo simulations utilizing the ABSINTH implicit solvation model, we show evidence for long-range interactions between polyQ tracts and both polyP regions. The polyQ segments promote the formation of *cis* peptide bonds within the polyP segment directly C-terminal to polyQ. This in turn will have important changes to the heterotypic interactions of htt through its polyP regions. A detailed comparative analysis of the monomer ensembles of N17-polyQ, polyQ-C38 and N17-polyQ-C38 constructs will be presented and the implications of our findings for loss- versus gain-of-function models for the onset and progression of disease will be discussed.

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Modulation of Amyloid Beta Peptide Aggregation by Apolipoprotein E Isoforms

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Apolipoprotein E4 (apoE) is a confirmed risk factor for late onset Alzheimer's disease (AD). Mouse model studies indicate enhanced depostion of amyloid beta (Aβ) peptides in the brain in presence of the ε4 isoform of apoE. However, the mechanism of apoE-Aβ interaction and the biophysical nature of these complexes remain largely unclear. In addition, the relative specificity of this interaction with the other two common isoforms (apoE2 and apoE3) not associated with AD is poorly understood. Aβ aggregation followed by turbidity measurements show that the aggregation is accelerated by all three isoforms of apoE and apoE4 showed the greatest and apoE2 the least effects. Fluorescence correlation spectroscopy (FCS) measurements using fluorescently labeled Aβ show that diffusion time of Aβ increases upon incubation with wild type apoE proteins. FCS data confirm that apoE4 has the highest and apoE2 has the lowest affinity for Aβ. However, this interaction appears to be slow and does not reach completion even after several days of co-incubation. The diffusion time of the complexes are found to be large indicating involvement of multimeric forms of Aβ. In addition, a monomeric form of apoE prepared by 4 mutations in the apoE sequence does not bind to Aβ. Thus apoE multimers may be acting as nucleation sites for oligomerization of Aβ. We are currently investigating the molecular basis of the apoE-Aβ complex formations by employing Hydrogen-Deuterium Exchange by mass spectrometry (HDX-MS).

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Novel Insights into Impairment of Membrane Integrity by Alpha-Synuclein Oligomers

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One of the most prevalent neurodegenerative diseases is Parkinson's disease, and is accompanied with the loss of dopaminergic neurons. Although the mechanisms leading to the death of these cells are still unclear, the protein alpha-synuclein is one of the pivotal factors. Previous studies indicate that especially oligomeric forms of alpha-synuclein show a detrimental effect on membrane integrity. As an intact membrane is crucial to many cellular processes, the impairment of the membrane integrity is a likely pathway for neuronal death. We use different phospholipid bilayer model systems to investigate the mechanisms underlying this process.

Using an approach based on the accessibility of a fluorescent probe integrated in the bilayer of phospholipid vesicles to a soluble quencher, we could show that the loss of fluorescence occurs by two different processes. Hereby, only